

**Manuscript Title: Assessment of energy availability and associated risk factors in professional female soccer players**

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## Abstract

This study aimed to assess energy availability (EA), alongside possible risk factors of reduced or low EA of professional female soccer players during a competitive season. Thirteen players (age:  $23.7 \pm 3.4$  y, stature:  $1.69 \pm 0.08$  m, body mass:  $63.7 \pm 7.0$  kg) engaged in a 5-day (two rest days, one light training, heavy training and match day) monitoring period. Energy intake (EI) and expenditure during exercise (EEE) were measured. EA was calculated and categorised as optimal, reduced or low ( $\geq 45$ , 31-44,  $\leq 30$  kcal·kg FFM<sup>-1</sup>·day<sup>-1</sup>, respectively). Relationships between EA and bone mineral density, resting metabolic rate (RMR), plasma micronutrient status, biochemical markers and survey data were assessed. EA was optimal for 15%, reduced for 62% and low for 23% of players. Higher EA was observed on rest days compared to others ( $P < 0.05$ ). EA was higher for the light compared to the heavy training day ( $P < 0.001$ ). EEE differed significantly between days ( $P < 0.05$ ). EI ( $2124 \pm 444$  kcal), carbohydrate ( $3.31 \pm 0.64$  g·kg·day<sup>-1</sup>) and protein ( $1.83 \pm 0.41$  g·kg·day<sup>-1</sup>) intake remained similar ( $P > 0.05$ ). Survey data revealed 23% scored  $\geq 8$  on the Low Energy Availability in Females Questionnaire and met criteria for low RMR (ratio  $< 0.90$ ). Relationships between EA and risk factors were inconclusive. Most players displayed reduced EA and did not alter EI or carbohydrate intake according to training or match demands. Although cases of low EA were identified, further work is needed to investigate possible long-term effects and risk factors of low and reduced EA separately to inform player recommendations.

## **Introduction**

The energetic demands of soccer, alongside the energy intake (EI) and diet composition of each player are key considerations especially during the season when competition is intensified (Thomas, Erdman, & Burke, 2017). Females playing in the top-flight national soccer league will routinely play one league match approximately every two weeks. In addition, teams that progress in the National cup and Champions' league are likely to play a competitive match every week (and in some cases twice per week). Therefore, a typical 7-day period for elite female soccer players may consist of 1-2 matches, 4-6 training sessions and a rest day. However, to date, how energy requirements might differ within a training and competition week for professional female soccer players is unknown.

Traditionally, energy balance (total EI minus total energy expenditure (EE)) has been a primary method used to assess energy requirements of athletes. However, energy balance has been criticised due to its inability to detect chronically undernourished athletes (for a review see Loucks, Kiens, & Wright, 2011). An alternative method to measure energy deficiency is to calculate energy availability (EA). EA is defined as the amount of energy that is available to support body functions, after subtracting the amount of energy that is expended during exercise and expressed relative to fat free mass (FFM). Importantly, EA is one of the three inter-related components of the Female Athlete Triad, along with menstrual health and bone health. It is known that chronic low energy availability (LEA) (with or without disordered eating) causes physiological alternations to conserve energy reserves, which have been highlighted in the International Olympic Committee statement on Relative Energy Deficiency in Sport (RED-S, Mountjoy et al., 2014). Specifically, there is suppression in metabolic and reproductive hormones which may lead to menstrual dysfunction (Loucks, 2003) and poor skeletal health (Kandemir et al., 2018). Low EI can also compromise macronutrient intake and micronutrient status, which have implications for player health and performance. In addition, a reduction in

resting metabolic rate (RMR) has been reported in athletes with LEA (De Souza, Hontscharuk, Olmsted, Kerr, & Williams, 2007).

Elite outfield female players typically cover ~10 km during a match, which mostly comprises walking or low-intensity running (~80%) alongside shorter periods of high-intensity exercise (Datson et al., 2014). Brewer (1994) reported exercise energy expenditure (EEE) of female players during professional match play as ~1100 kcal. More recently, Mara, Thompson and Pumpa (2015) reported EEE to be similar between match play (~644 kcal) and training (~607 kcal). Although these values were measured during pre-season training and a simulated friendly match (3 x 20 min periods), and therefore may not represent in-season training/match play. Only one study has assessed EA in elite female soccer players. In this study, Reed, De Souza and Williams (2013) monitored collegiate female players across a competitive season. Low energy availability (defined as  $<30 \text{ kcal} \cdot \text{kg FFM}^{-1} \cdot \text{day}^{-1}$ ) was observed in 26.3%, 33.3% and 11.8% of the team during the pre-, mid-, and post-season, respectively. However, for each of the time points, measurements of EA were collected for three consecutive days and the data was reported as a mean. Therefore, differences in EA between training (that differs in duration and intensity), match and rest days is not known.

Therefore, the aims of the current study were: (1) to measure EA of professional female soccer players during a light and heavy training day, two rest days and a competitive match; (2) to assess the macronutrient intake of elite female soccer players and (3) to investigate the relationship between EA and associated risk factors.

## **Methods**

### *Participants*

Thirteen professional female soccer players (mean  $\pm$  SD: age =  $23.7 \pm 3.4$  y, stature =  $1.69 \pm 0.08$  m, body mass  $63.7 \pm 7.0$  kg, starters  $n = 9$ ) provided written informed consent to participate in the study, after procedures were explained verbally and in writing. The study was approved by Loughborough University Research Ethics Committee, Loughborough, United Kingdom. The sample included eleven outfield players and two goalkeepers from the same team. Players competed in the top division of the Women's Super League (WSL1) and trained ~10 hrs per week.

### *Experimental design*

Up to two weeks prior to a 5-day monitoring period, players attended the testing facility between 0800 and 1000 h after an overnight fast to complete measurements of RMR and body composition (including BMD) and to provide a blood sample. Players were also asked to complete two questionnaires to assess symptoms relating to energy deficiency and eating-disorder behaviour, respectively.

All players then underwent a 5-day monitoring period to estimate energy intake (EI) and energy expenditure during exercise (EEE) to calculate daily and overall EA. This in-season (May) period comprised a heavy training day (day 1), a light training day (day 3), a competitive match day (day 4) and two rest days (days 2 and 5). The heavy day comprised two training sessions (1 x individualised resistance and 1 x field-based; distance covered:  $5897 \pm 1827$  m,  $63 \pm 17$  m/min<sup>-1</sup>), the light day comprised one training session (1 x field-based, distance covered:  $3201 \pm 336$  m,  $59 \pm 5$  m/min<sup>-1</sup>) while no sessions were scheduled for rest days. As this study aimed to monitor players in free-living conditions, no attempts were made to alter any aspect of the player's typical routine (i.e. training, competition or dietary habits).

### *Energy intake*

Total EI including food, fluid and supplement use was assessed using a 5-day weighed food diary. After written and verbal instruction, players were asked to record the quantity (using weighing scales), timing, preparation method and brand name of each item in writing. In an attempt to minimise the issues associated with estimating EI (Burke et al., 2018), the players also sent photographs at each mealtime and the researcher clarified any ambiguous information via direct questioning. Players were instructed to consume their typical diet throughout the monitoring period. During training days, players ate lunch *ad libitum* from a buffet but organised their own meals on all other occasions. Total EI and macronutrient composition were calculated using a commercially available nutrient analysis programme (Nutritics Ltd, Ireland). When items did not feature on the programme database, product labels were used for manual input. To omit between-researcher variability, all data were analysed by the lead researcher. To determine possible under- and over-reporters, the ratio between EI and RMR was calculated (Black, 2000), following which appropriate cut-off values were applied according to age and physical activity level (vigorous) (Black, 2000). Players with calculated values of EI:RMR in the interval 1.120 – 2.892 were classified as plausible reporters. Values outside the specified thresholds indicated under- and over-reporters. The recommendation to identify such instances but to include all records to avoid unknown bias in small sample sizes was adhered to (Black, 2000).

#### *Resting metabolic rate (RMR)*

RMR was measured via indirect calorimetry using the FitMate™ metabolic system (Cosmed, Cosmed, Rome, Italy), under overnight fasting conditions. Players were instructed to minimise physical activity before the test and abstain from caffeine 24 h prior to the test. Exercise was avoided for ~17 hrs prior to testing. Upon arrival and after voiding, body mass was measured (Seca, 813, Hamburg, Germany) and players laid still in a supine position wearing a facemask (Hans Rudolph, Kansas City, USA) attached to the device, for 10 min in a thermo-neutral (22

$\pm 1$  °C) dark room. After calibration, oxygen consumption was measured continuously for 15 min, with the final 10 min used to calculate RMR. Throughout testing, players were asked to breathe normally, avoid talking, limit movement and remain awake. The FitMate™ is reliable ( $r = 0.94-0.99$ ) and valid when assessed against the Douglas Bag method ( $r = 0.97$ , difference 5.81 kcal/day) (Nieman et al., 2006). To determine players with a low RMR, the ratio between the measured ( $mRMR$ ) and predicted RMR ( $pRMR$ ) was calculated using the Cunningham equation (1980), whereby a value  $<0.90$  was indicative of low (De Souza et al., 2008).

#### *Energy expenditure during exercise*

Training logs (including activity, exercise duration and rest periods) for resistance and any non-club based sessions were completed and assigned a Metabolic Equivalent (MET) value from the compendium of physical activities (Ainsworth et al., 2011). Values were corrected for individual variation using measured RMR, whereby energy expended for RMR for the duration of exercise was subtracted from the estimated EEE (Kozey, Lyden, Staudenmayer, & Freedson, 2010). For field-based sessions, players wore the same portable Global Positioning device (Viper Units, STATSports, Newry, Ireland), as previously described (Anderson et al., 2016) with individualised descriptive information inserted into the software. The estimated EEE was derived from the manufacturer's software and corrected for individual variation, as previously stated.

#### *Energy availability*

EI and EEE (kcal) over the 5-day monitoring period was used to calculate EA, where  $EA = (EI - EEE) / FFM$  (Loucks, 2004), and FFM is fat free mass. Energy availability was categorised as optimal ( $\geq 45 \text{ kcal} \cdot \text{kg FFM}^{-1} \cdot \text{day}^{-1}$ ), reduced ( $30-44 \text{ kcal} \cdot \text{kg FFM}^{-1} \cdot \text{day}^{-1}$ ) and low ( $<30 \text{ kcal} \cdot \text{kg FFM}^{-1} \cdot \text{day}^{-1}$ ) (Loucks et al., 2011).

### *Body composition*

Dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy Advance, GE Healthcare Encore version 14.00.439) was used to determine total mass (kg), body fat percent (%), total fat mass (kg), total lean mass (kg), total BMD (g/cm<sup>2</sup>), bone mineral content (kg) and *t*-score for each player. Calibration was conducted in line with manufacturer's guidelines with quality assurance checks carried out daily. The same technician conducted all scans, which were set on standard mode (16-25 cm). Reliability was referenced as statistically 68% of repeat scans fall within 1SD (+/- 0.8% Fat, +/- 210g Tissue Mass, +/- 520g Mass, +/- 610g Lean Mass for Total Body Total). All players wore training kit and removed jewellery before being scanned. The centrally standard position technique on the scanning pad was used. Normal BMD was classified as a Z-score >-1.0 (Nattiv et al., 2007).

### *Biochemical analysis*

A fasted blood sample was drawn from the antecubital vein while players remained seated. All samples were sent to a UKAS accredited commercial pathology company for analysis. Blood was separated and tested within 2 hrs. Analysis of micronutrients and biochemical markers (calcium, ferritin, serum iron, active B12, serum folate, vitamin D, magnesium, transferrin, transferrin saturation (%), free T3, insulin, glucose, cortisol, free thyroxine, thyroid stimulating hormone) were completed using the Siemens Allelica. Analysis of haemoglobin was performed using the Sysmex 1000i and IGF-1 using the Siemens Immulite.

### *The Low Energy Availability in Females Questionnaire (LEAF-Q)*



The LEAF-Q (Melin et al., 2014) was used to assess the prevalence of conditions associated with long-term energy deficiency. The 25-item self-report questions focus on symptoms related to injury, gastrointestinal (GI) and menstrual function over the past year. A global score  $\geq 8$  has been used to identify athletes at long-term risk of energy deficiency (Melin et al., 2014).

### *Eating pathology*

The Eating Disorder Examination –Questionnaire (EDE-Q) was used to discriminate between normal and pathological eating behaviour and full properties have been previously described elsewhere (Fairburn & Beglin, 2008). A score of 2.3 on the global scale, in conjunction with occurrence of any objective bulimic episodes and/ or use of excessive exercise for weight control, provides a positive predictive value of 0.56 (specificity = 0.96 and sensitivity = 0.83) (Mond, Hay, Rodgers, Owen, & Beumont, 2004). It has been demonstrated that the EDE-Q has acceptable to high internal consistency and test-retest reliability (Berg, Peterson, Frazier, & Crow, 2012).

### *Statistical analysis*

Statistical analysis was completed using SPSS version 21.0 (SPSS, Inc., Chicago, IL, USA) with the alpha level set at  $P \leq 0.05$ . Assessment of normality and sphericity (Mauchly's test,  $P \leq 0.05$ ) were completed, with any violations adjusted by the Greenhouse-Geisser correction. One way repeated-measures analysis of variance (ANOVA) were conducted to assess any differences between days for EA, EI, EEE and macronutrient intake. To assess any relationship between EA and possible associated variables (RMR, micronutrient status, biochemical markers, LEAF-Q score, bone mineral density, EI, EEE and macronutrient intake), Pearson bivariate correlations were conducted.

## **Results**

### *Validity of dietary records*

Twelve players met the criteria for plausible reporters (Black, 2000) and one player was identified as an under-reporter (EI:RMR = 1.09). As removing participants from the analysis of small sample sizes can create unknown bias, recommendations to include all records was adhered to (Black, 2000). Although all food diaries were analysed by the lead researcher, the variability in estimation from our laboratory is included for reference. Coefficient of variation is  $5.83 \pm 2.24\%$ ,  $5.58 \pm 7.11\%$ ,  $2.00 \pm 0.59\%$  and  $7.32 \pm 9.26\%$  for energy intake (kcal) and carbohydrate, protein and fat (g), respectively.

### *Energy availability*

Mean EA over the 5-day period was optimal for 15%, reduced for 62% and low for 23% of players. Significant differences in EA between days were observed ( $F(1.425, 17.104) = 9.868$ ,  $P=0.003$ ) (Figure 1). EA was greater on rest days compared to light, ( $P=0.004$ ) heavy ( $P<0.001$ ) and match day ( $P=0.008$ ). Significantly higher EA was found on the light compared to the heavy training day ( $P<0.001$ ; Table 1). However, there was no significant difference in EA when match day was compared to both light and heavy training days ( $P>0.05$ ).

\*\*\*Insert Figure 1 here\*\*\*

### *Energy intake*

No significant differences were found between days for total EI (kcal) ( $F(1.921, 23.047) = 0.833$ ,  $P>0.05$ ; Table 1). There were no between day differences in total carbohydrate ( $\text{g}\cdot\text{kg}^{-1}$ ) ( $F(3, 36) = 0.891$ ,  $P>0.05$ ) and protein ( $\text{g}\cdot\text{kg}^{-1}$ ) intake ( $F(3, 36) = 0.786$ ,  $P>0.05$ ). A main effect was found for fat intake ( $\text{g}\cdot\text{kg}^{-1}$ ) ( $F(3, 36) = 3.730$ ,  $P=0.02$ ), which was higher on the heavy day compared to the light training day ( $P=0.04$ ; Table 1). Mean carbohydrate intake over the 5-day

period was  $< 5 \text{ g}\cdot\text{kg}\cdot\text{day}^{-1}$  for all players. In addition, 46% of players consumed  $< 3 \text{ g}\cdot\text{kg}\cdot\text{day}^{-1}$ . More players (62%) consumed  $> 3 \text{ g}\cdot\text{kg}\cdot\text{day}^{-1}$  of carbohydrate on the heavy training day compared to all other days (39%).

On average, the majority of players (92%) consumed  $\geq 1.2 \text{ g}\cdot\text{kg}\cdot\text{day}^{-1}$  of protein. The percentage of players consuming  $< 1.2 \text{ g}\cdot\text{kg}\cdot\text{day}^{-1}$  of protein was similar between days (8-15%). For 39% of players, fat intake over the 5-day period was  $< 20$  -35% of total EI. All between-day comparisons can be found in Table 1.

\*\*\*Insert Table 1 here\*\*\*

#### *Energy expenditure during exercise*

There was a significant main effect for EEE over the 5-day monitoring period ( $F(1.145, 36) = 39.665, P < 0.001$ ), with lower EEE on rest days ( $15 \pm 54 \text{ kcal}$ ) compared to both light ( $299 \pm 78 \text{ kcal}$ ) and heavy ( $786 \pm 159 \text{ kcal}$ ) training days and the match day ( $881 \pm 473 \text{ kcal}$ ) (all  $P < 0.001$ ). EEE was also significantly lower during the light training day, compared to heavy training and match days ( $P < 0.001$ ) (Table 1).

#### *Body composition and resting metabolic rate*

Table 2 provides individual body composition, BMD and RMR information. All players had BMD Z-scores  $> -1.0$  (normal; Nattiv et al., 2007). Three players (23%) scored  $< 0.90$  for  $m\text{RMR} : p\text{RMR}$  (Cunningham, 1980).

\*\*\*Insert Table 2 here\*\*\*

#### *Questionnaires*

The LEAF-Q responses revealed a mean score of  $6.2 \pm 3.2$ , with 23% of players scoring  $\geq 8$ . Table 3 reveals additional information relative to the variables assessed on the questionnaire.

\*\*\*Insert Table 3 here\*\*\*

The EDE-Q responses revealed a mean score of  $0.57 \pm 0.68$ , with no player reaching the specified 2.3 cut-off (Mond et al., 2004). This global score, alongside all subscale scores (restraint  $0.60 \pm 0.89$ ; eating concern  $0.27 \pm 0.39$ ; shape concern  $0.76 \pm 1.14$ ; and weight concern  $0.76 \pm 1.14$ ) were below population norms, based on young adult women (Mond, Hay, Rodgers, & Owen, 2006).

#### *Micronutrient/ biochemical profile*

All micronutrient and biochemical profiles can be found in Table 2. For most micronutrients players achieved the recommended range. Values were below normal for Transferrin (for 50% of players) and Vitamin D (20%), whereas Iron was higher than normal values for 10% of players. Players were below the normal range for Free T3 and transferrin saturation (both 10%) but above the normal range for free thyroxine (all 10%) and cortisol (50%).

#### *Relationship between energy availability and associated risk factors*

Significant correlations were revealed between EA and: energy intake ( $r=0.87$ ,  $P<0.001$ ), protein intake ( $r=0.79$ ,  $P=0.001$ ) and EEE ( $r=-0.79$ ,  $P=0.001$ ). All relationships between EA and biochemical markers are presented in Table 2.

## **Discussion**

This study is the first to investigate differences in EA during a 5-day competitive period in professional female soccer players. The main finding of the study was that 85% of players did

not achieve a status of “optimal” for EA over the 5-day period. The proportion of players presenting LEA ( $<30 \text{ kcal}\cdot\text{kg FFM}^{-1}\cdot\text{day}^{-1}$ ) was highest on the heavy training day (69%) and match day (54%) but was less evident during light training day (38%) and rest days (0%).

Using the specified criteria (Loucks et al., 2011), results revealed 85% of players did not ingest sufficient energy to meet the demands of exercise, relative to their fat-free mass. While the majority (62%) of players presented reduced EA, there were also 23% (3/13) who met the criteria for LEA. These results are similar to Reed et al. (2013) who reported that 33.3% (5/15) of elite players were in LEA during the mid-season. However, Reed et al. (2013) grouped players based on ‘low’ ( $<30 \text{ kcal}\cdot\text{kg FFM}^{-1}\cdot\text{day}^{-1}$ ) or ‘higher’ EA ( $>30 \text{ kcal}\cdot\text{kg FFM}^{-1}\cdot\text{day}^{-1}$ ), meaning that it was not possible to distinguish between those in reduced (30-44  $\text{kcal}\cdot\text{kg FFM}^{-1}\cdot\text{day}^{-1}$ ) or optimal EA (Loucks, 2004). This is relevant as disruption in body functions could occur at unique EA “thresholds” such as when it is reduced, not just low (Burke et al., 2018). For example, our study showed that two of the three players with low RMR (ratio  $<0.90$ ) had reduced EA ( $34 \pm 3 \text{ kcal}\cdot\text{kg FFM}^{-1}\cdot\text{day}^{-1}$ ), which could reflect metabolic adaptation to prolonged energy deficiency (Cunningham, 1980). Abnormal menstrual function (using LEAF-Q) was reported by one player who was in LEA ( $19 \text{ kcal}\cdot\text{kg FFM}^{-1}\cdot\text{day}^{-1}$ ). Insufficient EA can lower leptin levels, which disrupts the secretion of luteinizing hormone pulses into the blood, therefore reducing the energy expended from menstruation (Loucks et al., 2011). However, the finding that LEA was apparent in some players without disruptions in menstrual function suggests that prolonged energy deficiency was not apparent in these cases. The low number of soccer players reporting menstrual dysfunction is in-line with other studies (Mullinix, Jonnalagadda, Rosenbloom, Thompson, & Kicklighter, 2002; Sundgot-Borgen & Torstveit, 2007).

Players achieved higher EA on rest days ( $42 \pm 7 \text{ kcal} \cdot \text{kg FFM}^{-1} \cdot \text{day}^{-1}$ ) than match and training days (29 to 35  $\text{kcal} \cdot \text{kg FFM}^{-1} \cdot \text{day}^{-1}$ ), accompanied with a negative relationship between EEE and EA ( $r=-0.79$ ,  $P=0.001$ ). This relationship was unsurprising owing to the use of EEE to calculate EA. Of note was the increasing number of players in LEA as the EEE increased, which is in line with the findings by Nattiv et al. (2007). For example, on the rest day, none of the players were in LEA, which increased to 38%, 54% and 69% on the light training, match day and heavy training day, respectively. This indicates that a high proportion of players failed to alter EI in response to the changes in daily exercise demands, evidenced by the minimal variation of EI between days ( $2031 \pm 548$  to  $2200 \pm 471 \text{ kcal} \cdot \text{day}^{-1}$ ).

Ensuring adequate carbohydrate availability to support exercise demands is a key requirement for soccer players (Burke, Loucks, & Broad, 2006). Furthermore, low carbohydrate availability is recognised as a key limiting factor for reproductive function and skeletal health (Loucks, 2004). The carbohydrate intake ( $3.31 \pm 0.64 \text{ g} \cdot \text{kg} \cdot \text{day}^{-1}$ ) was lower than previously reported in elite females ( $4.1 - 4.7 \text{ g} \cdot \text{kg} \cdot \text{day}^{-1}$ ; Martin, Lambeth, & Scott, 2006; Mullinix, Jonnalagadda, Rosenbloom, Thompson, & Kicklighter, 2003) and was below ACSM recommendations for moderate training ( $5-7 \text{ g} \cdot \text{kg} \cdot \text{day}^{-1}$ ) (Thomas et al., 2017). The results of the present study revealed that 92% of players ingested  $<5 \text{ g carbohydrate} \cdot \text{kg} \cdot \text{day}^{-1}$  for the heavy training day. However, the substantially lower intakes ( $<3 \text{ g} \cdot \text{kg} \cdot \text{day}^{-1}$ ) reported by many players on the heavy training (62%) and match day (39%) could result in insufficient glycogen availability and reduced performance in training and matches (Williams & Rollo, 2015). It was also notable that carbohydrate intake did not change significantly between days, despite differences in exercise demands. This is different to professional male players who were found to periodize carbohydrate to favour match compared to training days (Anderson et al., 2016).

Mean fat intake was lower than previously reported in elite female soccer (Martin et al., 2006; Mullinix et al., 2003). Fat intake warrants attention since chronic restriction may reduce the uptake of essential nutrients, although this was not evident for the micronutrients assessed in the present study (Table 2). However, vitamin D (25(OH)D) and transferrin were below recommended values for 20% and 50% of players, respectively. Insufficient ( $< 50$  nmol/l) vitamin D status may be due to sub-optimal dietary intake as these players also presented either low or reduced EA. Although players were tested during British Summer Time (May) it is also possible that low levels of sunlight exposure (from covering arms and legs during training) could have impeded vitamin D homeostasis (Owens, Allison, & Close, 2018). As players with low transferrin values did not present insufficiency in more than one marker of overall iron status (Iron and Ferritin), deficiency is unlikely (Sim et al., 2019). Consistent with previous studies (Martin et al., 2006; Mullinix et al., 2003; Reed, De Souza, Kindler, & Williams, 2014), the majority (92%) of players met protein recommendations ( $>1.2$  g  $\cdot$  kg $\cdot$  day $^{-1}$ ) over the 5-day period.

The diagnosis and management of reduced EA and LEA should consider recognised screening tools, with appropriate sensitivity and specificity (Burke et al., 2018). Findings from the EDE-Q suggested that players were not advertently reducing their energy intake, with scores below population norms (young adult women) (Mond et al., 2006). This was accompanied by a non-significant relationship between EA and the total questionnaire score ( $r = 0.44$ ). Findings were different to Reed et al. (2013) who reported a negative association between EA and both body dissatisfaction ( $r = -0.62$ ) and drive for thinness ( $r = -0.55$ ) in elite female soccer players. Similar studies also evidence eating disorders (prevalence = 24%, Sundgot-Borgen & Torstveit, 2007) and highlight players ‘at risk’ (prevalence = ~8%, Prather et al., 2016). Although not problematic in our group of players, the results from other studies, combined

with the observation of low carbohydrate intake would suggest that attitudes towards certain foods may be important when addressing LEA.

Bone mineral density was within the normal range for athletes (Mountjoy et al., 2014; Nattiv et al., 2007) for all players ( $z$ -scores: 1.1 to 3.8). This suggests that acute EA status did not compromise this element of the Female Athlete Triad. The observed values likely reflect the high level of mechanical loading in sports such as soccer, which is thought to slow down or reverse bone loss (Sundgot-Borgen & Torstveit, 2007). The lack of associations between EA and “potential” biochemical markers of EA (Table 2), contributes to the current conflicting evidence about their potential to monitor energy status and detect players ‘at risk’ of LEA based on a single measurement (Logue et al., 2018). Speculatively, these biomarkers may not be sensitive to short-term alterations in EA in individuals reporting normal menstruation.

Survey data from the LEAF-Q showed that 23% ( $n=3$ ) scored  $\geq 8$ , which has been used as cut-off criteria for being ‘at risk’ of a long-term energy deficiency (Melin et al., 2014). However, caution should be applied when interpreting the LEAF-Q data as the present study found a non-significant relationship ( $r = 0.22$ ) between EA and the LEAF-Q scores. It is possible that high scores on the injury subscale ( $6 \pm 1$ ) for ‘at risk’ players partially account for these results. Therefore, total scores above the cut-off ( $\geq 8$ ) could be due to the nature of soccer (i.e. contact) rather than problems with reduced EA or LEA *per se*. These results suggest that practitioners should use a combination of measures to screen for energy deficiency.

A limitation of the present study was that data collected only provides a “snapshot” assessment of player’s EA and thus may not represent long-term EA. The possibility that players altered



their dietary habits also cannot be overlooked. While nutrition staff confirmed that players were not following specific nutrition programmes to alter body composition, it is conceivable that some players might have been trying to achieve private individual goals, which could not be accounted for. Although food-diary analysis revealed that only one player was categorised as an under-reporter (Black, 2000), limitations of using these equations at the individual-level are evident and difficulties in assessment of EA are well-known (Burke et al., 2018). Moreover, due to the small sample size, it must be noted that results might not reflect other professional female soccer players.

In conclusion, the EA of elite female soccer players was low for 23% of players, and reduced for 62% of elite female soccer players during a 5-day in-season period. A high proportion of players did not modify their total energy intake or carbohydrate intake to the changing demands of training or competition. Although relationships between EA and associated risk factors suggested no negative health implications, player/s with low RMR and menstrual irregularities had reduced EA or LEA. However, due to the small sample size, further work is needed to investigate the implications of LEA and reduced EA separately to inform recommendations for professional female players.

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## References

- Ainsworth, B. E., Haskell, W. L., Herrmann, S. D., Meckes, N., Bassett, D. R., Jr., Tudor-Locke, C., . . . Leon, A. S. (2011). 2011 Compendium of Physical Activities: a second update of codes and MET values. *Med Sci Sports Exerc*, 43(8), 1575-1581. doi:10.1249/MSS.0b013e31821ece12
- Anderson, L., Orme, P., Di Michele, R., Close, G. L., Morgans, R., Drust, B., & Morton, J. P. (2016). Quantification of training load during one-, two- and three-game week schedules in professional soccer players from the English Premier League: implications for carbohydrate periodisation. *J Sports Sci*, 34(13), 1250-1259. doi:10.1080/02640414.2015.1106574
- Anderson, L., Orme, P., Di Michele, R., Close, G. L., Morgans, R., Drust, B., & Morton, J. P. (2016). Quantification of training load during one-, two- and three-game week schedules in professional soccer players from the English Premier League: implications for carbohydrate periodisation. *Journal of Sports Sciences*, 34(13), 1250-1259. doi:10.1080/02640414.2015.1106574
- Berg, K. C., Peterson, C. B., Frazier, P., & Crow, S. J. (2012). Psychometric evaluation of the eating disorder examination and eating disorder examination-questionnaire: a systematic review of the literature. *Int J Eat Disord*, 45(3), 428-438. doi:10.1002/eat.20931
- Black, A. E. (2000). Critical evaluation of energy intake using the Goldberg cut-off for energy intake : basal metabolic rate. A practical guide to its calculation, use and limitations. *International Journal of Obesity*, 24(9), 1119-1130. doi:10.1038/sj.ijo.0801376
- Brewer, J. (1994). Nutritional aspects of women's soccer. *J Sports Sci*, 12 Spec No, S35-38.
- Burke, L. M., Loucks, A. B., & Broad, N. (2006). Energy and carbohydrate for training and recovery. *Journal of Sports Sciences*, 24(7), 675-685. doi:10.1080/02640410500482602
- Burke, L. M., Lundy, B., Fahrenholtz, I. L., & Melin, A. K. (2018). Pitfalls of Conducting and Interpreting Estimates of Energy Availability in Free-Living Athletes. *International Journal of Sport Nutrition and Exercise Metabolism*, 28(4), 350-363. doi:10.1123/ijsnem.2018-0142
- Cunningham, J. J. (1980). A reanalysis of the factors influencing basal metabolic-rate in normal adults. *American Journal of Clinical Nutrition*, 33(11), 2372-2374. doi:10.1093/ajcn/33.11.2372
- Datson, N., Hulton, A., Andersson, H., Lewis, T., Weston, M., Drust, B., & Gregson, W. (2014). Applied Physiology of Female Soccer: An Update. *Sports Medicine*, 44(9), 1225-1240. doi:10.1007/s40279-014-0199-1
- De Souza, M. J., Hontscharuk, R., Olmsted, M., Kerr, G., & Williams, N. I. (2007). Drive for thinness score is a proxy indicator of energy deficiency in exercising women. *Appetite*, 48(3), 359-367. doi:10.1016/j.appet.2006.10.009
- De Souza, M. J., West, S. L., Jamal, S. A., Hawker, G. A., Gundberg, C. M., & Williams, N. I. (2008). The presence of both an energy deficiency and estrogen deficiency exacerbate alterations of bone metabolism in exercising women. *Bone*, 43(1), 140-148. doi:10.1016/j.bone.2008.03.013
- Fairburn, C. G., & Beglin, S. (2008). *Cognitive behavior therapy and eating disorders* (C. G. Fairburn Ed.). Guildford Press.
- Kandemir, N., Slattery, M., Ackerman, K. E., Tulsiani, S., Bose, A., Singhal, V., . . . Misra, M. (2018). Bone Parameters in Anorexia Nervosa and Athletic Amenorrhea:

- Comparison of Two Hypothalamic Amenorrhea States. *J Clin Endocrinol Metab*, 103(6), 2392-2402. doi:10.1210/jc.2018-00338
- Kozey, S., Lyden, K., Staudenmayer, J., & Freedson, P. (2010). Errors in MET Estimates of Physical Activities Using  $3.5 \text{ ml.kg}^{-1}.\text{min}^{-1}$  as the Baseline Oxygen Consumption. *Journal of Physical Activity & Health*, 7(4), 508-516. doi:10.1123/jpah.7.4.508
- Logue, D., Madigan, S. M., Delahunt, E., Heinen, M., Mc Donnell, S.-J., & Corish, C. A. (2018). Low Energy Availability in Athletes: A Review of Prevalence, Dietary Patterns, Physiological Health, and Sports Performance. *Sports Medicine*, 48(1), 73-96. doi:10.1007/s40279-017-0790-3
- Loucks, A. B. (2003). Energy availability, not body fatness, regulates reproductive function in women. *Exerc Sport Sci Rev*, 31(3), 144-148.
- Loucks, A. B. (2004). Energy balance and body composition in sports and exercise. *J Sports Sci*, 22(1), 1-14. doi:10.1080/0264041031000140518
- Loucks, A. B., Kiens, B., & Wright, H. H. (2011). Energy availability in athletes. *Journal of Sports Sciences*, 29, S7-S15. doi:10.1080/02640414.2011.588958
- Mara, J. K., Thompson, K. G., & Pumpa, K. L. (2015). Assessing the energy expenditure of elite female soccer players: A preliminary study. *Journal of Strength and Conditioning Research*, 29(10), 2780-2786. doi:10.1519/jsc.0000000000000952
- Martin, L., Lambeth, A., & Scott, D. (2006). Nutritional practices of national female soccer players: Analysis and recommendations. *Journal of Sports Science and Medicine*, 5(1), 130-137.
- Melin, A., Tornberg, A. B., Skouby, S., Faber, J., Ritz, C., Sjodin, A., & Sundgot-Borgen, J. (2014). The LEAF questionnaire: a screening tool for the identification of female athletes at risk for the female athlete triad. *Br J Sports Med*, 48(7), 540-545. doi:10.1136/bjsports-2013-093240
- Mond, J. M., Hay, P. J., Rodgers, B., & Owen, C. (2006). Eating Disorder Examination Questionnaire (EDE-Q): norms for young adult women. *Behav Res Ther*, 44(1), 53-62. doi:10.1016/j.brat.2004.12.003
- Mond, J. M., Hay, P. J., Rodgers, B., Owen, C., & Beumont, P. J. (2004). Validity of the Eating Disorder Examination Questionnaire (EDE-Q) in screening for eating disorders in community samples. *Behav Res Ther*, 42(5), 551-567. doi:10.1016/S0005-7967(03)00161-X
- Mountjoy, M., Sundgot-Borgen, J., Burke, L., Carter, S., Constantini, N., Lebrun, C., . . . Ljungqvist, A. (2014). The IOC consensus statement: beyond the Female Athlete Triad-Relative Energy Deficiency in Sport (RED- S). *British Journal of Sports Medicine*, 48(7), 491-+. doi:10.1136/bjsports-2014-093502
- Mullinix, M. C., Jonnalagadda, S. S., Rosenbloom, C. A., Thompson, W. R., & Kicklighter, J. R. (2002). Dietary intake and health status of elite female US soccer players. *Faseb Journal*, 16(4), A252-A252.
- Mullinix, M. C., Jonnalagadda, S. S., Rosenbloom, C. A., Thompson, W. R., & Kicklighter, J. R. (2003). Dietary intake of female US soccer players. *Nutrition Research*, 23(5), 585-593. doi:10.1016/s0271-5317(03)00003-4
- Nattiv, A., Loucks, A. B., Manore, M. M., Sanborn, C. F., Sundgot-Borgen, J., Warren, M. P., & American College of Sports, M. (2007). American College of Sports Medicine position stand. The female athlete triad. *Med Sci Sports Exerc*, 39(10), 1867-1882. doi:10.1249/mss.0b013e318149f111
- Nieman, D. C., Austin, M. D., Benezra, L., Pearce, S., McInnis, T., Unick, J., & Gross, S. J. (2006). Validation of Cosmed's FitMate in measuring oxygen consumption and estimating resting metabolic rate. *Res Sports Med*, 14(2), 89-96. doi:10.1080/15438620600651512

- Owens, D. J., Allison, R., & Close, G. L. (2018). Vitamin D and the Athlete: Current Perspectives and New Challenges. *Sports Medicine*, 48, S3-S16. doi:10.1007/s40279-017-0841-9
- Prather, H., Hunt, D., McKeon, K., Simpson, S., Meyer, E. B., Yemm, T., & Brophy, R. (2016). Are Elite Female Soccer Athletes at Risk for Disordered Eating Attitudes, Menstrual Dysfunction, and Stress Fractures? *Pm&R*, 8(3), 208-213. doi:10.1016/j.pmrj.2015.07.003
- Reed, J. L., De Souza, M. J., Kindler, J. M., & Williams, N. I. (2014). Nutritional practices associated with low energy availability in Division I female soccer players. *Journal of Sports Sciences*, 32(16), 1499-1509. doi:10.1080/02640414.2014.908321
- Reed, J. L., De Souza, M. J., & Williams, N. I. (2013). Changes in energy availability across the season in Division I female soccer players. *J Sports Sci*, 31(3), 314-324. doi:10.1080/02640414.2012.733019
- Sim, M., Garvican-Lewis, L. A., Cox, G. R., Govus, A., McKay, A. K. A., Stellingwerff, T., & Peeling, P. (2019). Iron considerations for the athlete: a narrative review. *Eur J Appl Physiol*, 119(7), 1463-1478. doi:10.1007/s00421-019-04157-y
- Sundgot-Borgen, J., & Torstveit, M. K. (2007). The female football player, disordered eating, menstrual function and bone health. *British Journal of Sports Medicine*, 41, I68-I72. doi:10.1136/bjsm.2007.038018
- Thomas, D. T., Erdman, K.A., & Burke, L. (2017). Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and Athletic Performance (vol 116, pg 521, 2016). *Journal of the Academy of Nutrition and Dietetics*, 117(1), 146-146. doi:10.1016/j.jand.2016.11.008
- Williams, C., & Rollo, I. (2015). Carbohydrate Nutrition and Team Sport Performance. *Sports Med*, 45 Suppl 1, S13-22. doi:10.1007/s40279-015-0399-3

## Captions

**Figure 1.** The relative percentage of players in optimal (dotted bars), reduced (striped bars) and low (black bars) energy availability for rest days (average of 2 days), a light training day (x 1 session), a heavy training day (average of 2 sessions) and a match day, where optimal EA is  $\geq 45$  kcal/ kg FFM/ day, reduced EA is  $\geq 30$ -44 kcals/ kg FFM/ day and low EA is  $\leq 30$  kcals/ kg FFM/ day (Loucks et al., 2004;  $n = 13$ ). \* indicates significant difference to the rest day and ^ indicates significant difference to the light training day ( $P < 0.05$ ).

**Table 1.** Energy availability, energy intake, energy expenditure and macronutrient intakes ( $n = 13$ , mean  $\pm$  SD)

**Table 2.** Descriptive information for body composition, resting metabolic rate ( $n=13$ ), micronutrient and biochemical profiles of players ( $n=10$ ) and their association with energy availability.

**Table 3.** Responses to the LEAF-Q, including injury, contraception, menstrual function, and gastrointestinal function